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# Review on Methodological Advances in Multi-Spectral Analysis: Mechanics, Optical Layouts, and Algorithmic Deconvolution Workflows for UV-Visible and Infrared Spectrophotometry

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**Abstract:** *The precise verification of structural configurations and molecular layouts constitutes a foundational pillar within analytical chemistry sectors and pharmaceutical validation settings. Among available options, Ultraviolet-Visible (UV-Vis) along with Infrared (IR) spectrophotometric modalities are highly favored owing to their speed, consistency, and ability to deliver complementary insights regarding electronic states and molecular vibrational modes. UV-Vis instruments primarily monitor variations in electronic energy levels, structural conjugation, and active chromophoric networks. Concurrently, IR protocols map individual functional arrangements by capturing quantized atomic displacements within covalent structures. Utilizing these two distinct approaches in tandem builds a highly reliable analytical matrix that substantially increases structural elucidation precision. This systematic review provides a rigorous breakdown of foundational wave mechanics, instrumental architectures—including single and dual-pathway optical systems—mathematical modeling techniques for overlapping drug mixtures, and step-by-step diagnostic workflows. Furthermore, practical analytical walkthroughs and green chemistry benefits are examined to offer an operational baseline for quality assurance professionals and academic researchers.*

**Keywords:** *Spectrophotometric Validation, Electronic Transitions, Molecular Displacement, Chromophore Mapping, Quantitative Mathematical Modeling, Analytical Architecture.*

## I. INTRODUCTION

Modern pharmaceutical exploration and chemical analysis rely heavily on instrumental protocols capable of identifying the precise composition, spatial layout, and purity profiles of target molecules. By systematically measuring how an isolated substance mitigates incoming bands of electromagnetic radiation, investigators can accurately determine functional connectivity, chemical bond strengths, and the central carbon skeleton of an unmapped sample.

Within standard quality control environments, UV-Vis and IR techniques serve as highly complementary, orthogonal pillars of discovery. UV-Vis spectrophotometric methods track the attenuation of radiation within ultraviolet boundaries (180–380 nm) and visible ranges (380–780 nm), revealing electronic excitations across conjugated pi systems and non-bonding electron pairs. Conversely, IR spectroscopy maps the underlying structural connectivity, functioning as a structural fingerprinting system that records discrete shifts in the vibrational energy states of covalent networks.

Because these techniques target distinct physical properties—UV-Vis focusing on low-level quantitation and conjugated frameworks, while IR isolates localized chemical bonds—merging their datasets yields a comprehensive structural narrative. This comprehensive review synthesizes foundational physics, modern instrument design improvements, and advanced algorithms to provide a structured, logical sequence for spectral deconvolution.

## II. FUNDAMENTAL PRINCIPLES OF UV-VISIBLE SPECTROSCOPY

### A. Core Operating Mechanism

The physical framework of UV-Vis spectrophotometry involves tracking changes in light intensity as specific wavelengths of ultraviolet or visible radiation are absorbed by an analyte. Exposing a sample to wavelengths spanning 180–780 nm causes incoming photons to transfer energy directly to valence electrons resting within ground-state molecular orbitals.

This energy transfer promotes an electron to a higher-energy, vacant antibonding orbital, provided the incident photon energy perfectly matches the gap between the two states.

This excitation occurs selectively within specific functional arrangements known as chromophores. The instrument records this reduction in light energy, processing it as absorbance or transmittance to generate the standard UV-Vis curve. The size of the electronic gap dictates the maximum absorption wavelength ( $\lambda_{\max}$ ):

smaller gaps require lower energy and shift absorption toward longer wavelengths, while a higher population of absorbing structures linearly increases total absorbance intensity.

### B. Categorization of Valence Electronic Transitions

Organic molecules contain bonding molecular orbitals ( $\sigma$  and  $\pi$ ), non-bonding atomic pathways containing unshared pairs ( $n$ ), and corresponding high-energy antibonding pathways ( $\sigma^*$  and  $\pi^*$ ). Because non-bonding electrons do not participate directly in chemical bonding, an  $n^*$  state does not exist. Inducing excitation pathways within these networks drives four primary electronic transitions:

- **Sigma to Sigma Star ( $\sigma \rightarrow \sigma^*$ ) Transitions:** These require high-energy, short-wavelength photons, typically below 150 nm. They occur exclusively within fully saturated systems containing stable single sigma bonds, such as alkanes, and must be analyzed using specialized vacuum-UV equipment operating below the 200 nm threshold.
- **N to Sigma Star ( $n \rightarrow \sigma^*$ ) Transitions:** Prevalent in saturated molecular frameworks containing heteroatoms that possess lone pairs, including alcohols, amines, and organohalides. These pathways require moderate energy inputs and produce bands within the far-UV region between 150 and 200 nm.
- **Pi to Pi Star ( $\pi \rightarrow \pi^*$ ) Transitions:** Highly common across unsaturated systems containing double or triple bonds, conjugated dienes, and multi-ring aromatic networks. These paths are extremely sensitive to structural adjustments and generate high-intensity absorption bands within standard quartz optical limits between 200 and 400 nm.
- **N to Pi Star ( $n \rightarrow \pi^*$ ) Transitions:** Occur when an unshared lone pair from a heteroatom is promoted into an empty antibonding pi orbital, a pattern typical of carbonyl, nitro, or thiocarbonyl arrangements. These require lower energy thresholds, yielding distinct but lower-intensity absorption troughs at longer wavelengths around 300 nm.

### C. The Governing Relation: Beer-Lambert Law

Low-level quantitation via UV-Vis methods relies directly on the Beer-Lambert Law. This relation states that when monochromatic light travels through a uniform, non-scattering solution, the total radiation absorbed scales linearly with both the concentration of the target chemical species and the optical path length of the sample holder. Mathematically, this is expressed as:  $A = \epsilon \cdot b \cdot c$ . Within this framework,  $A$  represents measured absorbance,  $\epsilon$  represents the molar absorptivity constant unique to that structure at a specific wavelength,  $b$  denotes the internal width of the sample cell, and  $c$  represents the molar concentration of the solution. This linear relationship forms the foundation of modern quantitative pharmaceutical validation protocols.

## III. OPTICAL SYSTEMS AND COMPONENT ASSEMBLIES FOR UV-VISIBLE EQUIPMENT

### A. Optical Geometric Classifications

Modern UV-Vis instruments are organized around three primary optical layouts. Single-Beam Spectrophotometers direct light sequentially from a continuous source through a focusing lens and a dispersion monochromator, passing directly through a single sample holder to reach the detector. Although cost-effective and mechanically straightforward, single-beam models require manual calibration against a baseline solvent blank before every run to compensate for source intensity drift over time.

Double-Beam Spectrophotometers integrate a dynamic beam splitter or spinning chopper paired with alternating mirror assemblies. This architecture divides the primary monochromatic light into two parallel pathways: one continuously monitors a reference blank, while the other simultaneously queries the analytical sample. The detector compares these signals continuously, correcting for source fluctuations and baseline drift to enable fully automated wavelength scans.

Photodiode Array (PDA) Spectrophotometers reverse the traditional layout by passing the complete polychromatic light beam directly through the sample cell before dispersion. The transmitted light then hits a fixed diffraction grating that projects the separated spectrum across a stationary array of hundreds of independent photodiodes.

This enables instantaneous, multi-wavelength detection, making it highly effective for rapid automated profiling and real-time chromatographic detection.

### B. Core Hardware Elements

To provide stable coverage across the entire analytical spectrum, instruments combine distinct light sources. Deuterium or hydrogen discharge lamps produce a steady continuum across the UV region (160–450 nm) via a high-voltage electrical arc. A tungsten-halogen filament lamp supplies radiation for the visible spectrum (330–900 nm), while high-pressure xenon flash lamps offer a versatile alternative covering 250–600 nm thresholds.

The monochromator mechanism isolates a narrow band of monochromatic wavelengths from these polychromatic sources. Incoming light enters through an entrance slit, is parallelized by a collimating mirror, and strikes a dispersive element—either a reflective diffraction grating or a quartz prism—which separates the light into its component wavelengths. A precision motor rotates this element so that only the selected wavelength passes through the exit slit to reach the sample.

Samples are measured inside high-purity optical cells known as cuvettes. Saturated visible assays can utilize inexpensive plastic or silicate glass; however, because silicate glass absorbs strongly below 350 nm, high-purity quartz cuvettes with a standardized 1 cm path length must be used for all UV-range analysis. The transmitted light strikes a high-sensitivity detector—such as a photomultiplier tube or a silicon photodiode—which converts the incoming light into a proportional electrical signal for quantitation.

## IV. MATHEMATICAL MODELING AND MULTI-COMPONENT DECONVOLUTION METHODS

When quantifying multi-component pharmaceutical mixtures, overlapping UV absorption profiles often obscure individual peak maxima. In such scenarios, analytical laboratories avoid tedious physical separation steps by applying specific mathematical models to deconvolve the complex, shared spectrum directly:

- 1) Simultaneous Equation Method (Vierordt's Method): Relies on the principle of absorbance additivity. By measuring total absorbances at two distinct peak maxima and calculating individual absorptivity constants, the exact concentrations of both components can be resolved using simultaneous linear equations.
- 2) Difference Spectrophotometry: Utilized when an analyte undergoes distinct, reversible structural shifts in response to pH alterations. The absorption profile of the drug in an acidic state is electronically subtracted from its basic state, isolating the target compound's signal from background excipient interferences.
- 3) Derivative Spectrophotometry: Converts standard zero-order spectra into first, second, or higher-order mathematical derivatives. This approach sharpens broad bands and separates overlapping peaks by locating zero-crossing points where an interfering substance's profile drops to zero, allowing independent measurement of the target analyte.
- 4) Absorbance Ratio Spectra Method: The raw spectrum of an unseparated mixture is mathematically divided by a standardized reference spectrum of one component, creating a ratio plot with plateau regions proportional solely to the remaining component's concentration.
- 5) Derivative Ratio Spectra Method: Combines ratio transformation with derivative steps. Calculating the mathematical derivative of an unaligned ratio spectrum removes baseline offsets and eliminates complex multicomponent interferences.
- 6) Double Divisor Ratio Spectra Derivative Method: Employs a paired mathematical divisor consisting of two standard interfering compounds simultaneously, resolving ternary (three-component) mixtures without requiring physical separation.
- 7) Successive Ratio-Derivative Spectra Method: Applies sequential mathematical divisions to a complex multicomponent spectrum, isolating individual concentration variables step by step.
- 8) Q-Absorbance Ratio Method: Relies on the ratio of absorbances at two specific wavelengths: the peak absorption maximum of one analyte and an isosbestic (isoabsorptive) point where both compounds exhibit identical absorptivity constants regardless of concentration.
- 9) Isosbestic Point Method: Identifies a precise, invariant wavelength where the molar extinction coefficients of two interconverting chemical states are perfectly identical, establishing an internal reference point for concentration monitoring.
- 10) Absorptivity Factor Method: Uses a single calibration constant derived from un-overlapped baseline sectors to mathematically eliminate overlapping spectral contributions from interfering matrices.

## V. ALGORITHMIC UV-VISIBLE INTERPRETATION: WORKFLOW AND VALIDATION WALK THROUGH

### A. Systematic Structural Analysis Protocol

To extract reliable structural configurations from an uncharacterized UV-Vis spectrum, analysts should follow this structured interpretation sequence: First, evaluate the overall shape of the wide spectrum scan, noting that broad, flat, or highly structured bands indicate specific classes of chemical compounds. Second, count the total number and track the intensity of individual absorption bands, which correlates directly with the variety of chromophores in the sample. Third, identify the absorbance magnitude and exact wavelength positions for the emerging peaks. Fourth, cross-reference these findings against reference data to propose likely chromophores. Fifth, observe any shifts in the absorption bands driven by adjacent substituents. Auxochromes (such as methyl, hydroxyl, alkoxy, halogen, and amino groups) can alter the position and intensity of a chromophore peak, triggering a bathochromic (red) shift, hypsochromic (blue) shift, hyperchromic effect (increased intensity), or hypochromic effect (decreased intensity). Finally, adjust the proposed framework to match the expected profile of candidate chemical compounds.

### B. Case Analysis Validation: Ethene Matrix

Applying this protocol to the verification of pure gaseous Ethene provides a clear case analysis. Resolving the spectrum yields a single absorption peak indicating the presence of an excited valence electron. The isolated peak reveals a sharp maximum located precisely between 160 and 165 nm. This short wavelength, high-energy profile corresponds to a standard localized pi to pi\* transition from an unconjugated double bond. Comparing this localized profile to empirical parameters confirms the presence of the basic alkene structure, validating the identity of Ethene.

## VI. FUNDAMENTAL PRINCIPLES OF INFRARED (IR) SPECTROSCOPY

### A. Wave Physics and Quantum Mechanics

Infrared spectroscopy identifies chemical structures by monitoring energy state variations within molecular bonds exposed to infrared radiation. Unlike electronic excitation, absorbing lower-energy infrared light causes bonded atoms to vibrate continuously around their equilibrium bond lengths. For a molecular vibration to absorb an infrared photon, the motion must generate a net change in the molecule's dipole moment. Consequently, highly symmetric molecules with non-polar bonds remain IR-inactive.

The standard IR spectrum is divided into three regions: Near-IR, Mid-IR, and Far-IR. The Mid-IR band (4000–400  $\text{cm}^{-1}$ ) serves as the primary zone for structural identification. Frequency is tracked using wavenumber ( $\nu$ ), defined as the reciprocal of wavelength in centimeters ( $\text{cm}^{-1}$ ), which scales linearly with energy. Spectral charts plot wavenumber along the X-axis (decreasing from left to right) against Percent Transmittance ( $T\%$ ) along the Y-axis. This causes absorption events to appear as inverted troughs, where zero transmittance indicates complete light absorption.

### B. Categories of Molecular Vibrations

The complex movements of atoms relative to one another are classified into two broad categories: Stretching and Bending vibrations. A stretching vibration involves a continuous change in interatomic distance along the shared bond axis between two atoms. This includes symmetrical stretching (attached atoms move simultaneously away from or toward the central atom along the bond axis) and asymmetrical stretching (one atom approaches the central atom while the other moves away).

Bending vibrations involve a change in the relative bond angle between two attached atoms without altering their individual bond lengths. These require lower energy inputs and appear at longer wavelengths (lower wavenumbers) than corresponding stretches. Bending vibrations are divided into in-plane bending (including scissoring, where atoms approach each other, and rocking, where they swing back and forth synchronously in the same plane) and out-of-plane bending (including wagging, where atoms oscillate up and down simultaneously above and below the molecular plane, and twisting, where they rotate in opposite directions out of the plane).

## VII. INSTRUMENTATION LAYOUTS AND SOLID PREPARATION PROTOCOLS FOR IR SYSTEMS

### A. Component Assembly

Modern infrared instruments require steady thermal emitters. Common options include the Globar (a silicon carbide rod) or the Nernst Glower (composed of fused rare-earth oxides like zirconium and yttrium). Standard glass or quartz optics cannot be used because they absorb strongly across the IR spectrum. Modern laboratories use Fourier Transform Infrared (FTIR) spectrophotometers built around a Michelson interferometer.

This configuration splits a light beam, passes it through a moving mirror mechanism to create an interferogram, and captures data across all wavenumbers simultaneously before decoding the signal via a fast Fourier transform algorithm. These signals are captured by sensitive thermal detectors, such as bolometers or thermocouples, which monitor internal potential or resistance changes triggered by the infrared beam.

#### B. Pellet Matrix Processing: Potassium Bromide Discs

Solid samples must be rendered optically transparent to prevent light scattering. The KBr Pressed Pellet Technique is an effective approach for analyzing solid materials. The analyst thoroughly grinds a small amount of the solid sample with roughly 100 times its weight of pure, dry potassium bromide powder. This mixture is loaded into a specialized steel die and subjected to mechanical pressure under a heavy press to form a thin, transparent disc (about 1–2 mm thick and 1 cm in diameter). This pellet is transparent to IR radiation and can be analyzed directly. This method avoids the interfering background bands generated by hydrocarbon mulling agents like Nujol.

### VIII. DIAGNOSTIC ALGORITHMIC PROTOCOL FOR IR INTERPRETATION

To decode an unknown FTIR spectrum, analysts use a structured, zone-based diagnostic sequence to identify or rule out candidate functional groups:

Step 1: Check the axes, baseline, and noise. Confirm the x-axis represents wavenumber in  $\text{cm}^{-1}$  (usually 4000–400  $\text{cm}^{-1}$ ) and the y-axis shows percent transmittance or absorbance. A crooked baseline or extreme noise signals preparation issues, such as a contaminated cell or poor pellet clarity. Identify major strong dips to isolate primary functional marks.

Step 2: Analysis of the O-H and N-H stretching regions (4000–2500  $\text{cm}^{-1}$ ). A very broad, intense peak centered between 3200 and 3600  $\text{cm}^{-1}$  confirms an alcohol or phenol O-H group with strong hydrogen bonding. Free hydroxyls appear sharper and narrower near 3600  $\text{cm}^{-1}$ . Amine or amide N-H stretches appear as sharper, less intense bands between 3300 and 3500  $\text{cm}^{-1}$ . Primary amines show a distinct doublet spike, secondary amines display a single peak, and amides are accompanied by a nearby strong carbonyl stretch.

Step 3: Analysis of C-H stretching and unsaturation. Inspect the peaks around the critical 3000  $\text{cm}^{-1}$  boundary line. Absorptions appearing strictly below 3000  $\text{cm}^{-1}$  (2850–2960  $\text{cm}^{-1}$ ) indicate saturated  $\text{sp}^3$  C-H bonds from alkanes. Presence of strong peaks shifting just above 3000  $\text{cm}^{-1}$  (3000–3100  $\text{cm}^{-1}$ ) establishes  $\text{sp}^2$  C-H stretching, confirming alkenes or aromatic rings. A terminal alkyne presents a sharp, isolated  $\text{sp}$  C-H band near 3300  $\text{cm}^{-1}$ .

Step 4: Analysis of the triple bond region (2500–2000  $\text{cm}^{-1}$ ). Scan this quiet region for sharp, narrow features. A distinct band near 2250  $\text{cm}^{-1}$  confirms a nitrile (C≡N) group, whereas a weak peak between 2100 and 2260  $\text{cm}^{-1}$  points to an alkyne (C≡C) framework.

Step 5: Analysis of the double bond and carbonyl region (2000–1500  $\text{cm}^{-1}$ ). The carbonyl (C=O) bond produces an exceptionally strong, sharp peak between 1650 and 1750  $\text{cm}^{-1}$ . Unconjugated ketones and aldehydes appear between 1715 and 1725  $\text{cm}^{-1}$ , with aldehydes showing additional small C-H shoulder peaks near 2720 and 2820  $\text{cm}^{-1}$ . Esters shift higher to 1735–1750  $\text{cm}^{-1}$ . Amides shift lower to 1650–1690  $\text{cm}^{-1}$  due to nitrogen resonance. Conjugation with an adjacent double bond or aromatic ring lowers the carbonyl frequency by 10–30  $\text{cm}^{-1}$ .

Step 6: Analysis of the fingerprint region (1500–400  $\text{cm}^{-1}$ ) and final structural integration. Examine this complex zone for unique molecular configurations. Look for strong C-O single-bond stretches between 1000 and 1300  $\text{cm}^{-1}$  to verify alcohols, ethers, or esters. Aromatic systems show sharp skeletal ring modes near 1600 and 1500  $\text{cm}^{-1}$ , coupled with diagnostic out-of-plane C-H bending patterns between 700 and 900  $\text{cm}^{-1}$  that reveal the exact substitution layout of the benzene ring. Combine all observations to ensure they tell a consistent structural story.

### IX. ANALYTICAL IMPLEMENTATIONS AND OPERATIONAL FRAMEWORKS

The parallel application of UV-Vis and IR spectrophotometry forms the backbone of several critical sectors. In qualitative analysis, IR is primarily deployed to identify unknown compounds, perform raw material verification, and confirm synthesis pathways by mapping functional groups. In contrast, UV-Vis is heavily favored for quantitative applications, including batch uniformity checks, dissolution testing, and active ingredient calculations in pharmaceutical formulations.

Furthermore, these modalities are increasingly integrated into online separation systems. UV-Vis acts as a premier detector for High-Performance Liquid Chromatography (HPLC) to profile column effluents in real time, while FTIR interfaces with gas chromatography or thermogravimetric systems to analyze volatile degradation products.

Finally, both techniques offer exceptional green chemistry advantages; when properly calibrated with multi-component mathematical models, they can analyze complex pharmaceutical mixtures directly from aqueous solutions, completely eliminating the need for toxic organic mobile phases required by conventional chromatography.

## X. CONCLUSION

UV-Visible and Infrared spectroscopy represent highly reliable, foundational tools for structural analysis. This review detailed how their underlying chemical physics can be integrated into systematic laboratory workflows. UV-Vis spectrophotometry remains excellent for low-level quantification and profiling conjugated electron networks, while FTIR acts as an effective screening method for identifying or ruling out functional groups based on their bond vibrations. Integrating multi-component mathematical models allows for the simultaneous determination of complex mixtures without requiring solvent-heavy physical separation steps. Maintaining strict structural formatting, utilizing robust diagnostic algorithms, and leveraging the orthogonal strengths of both techniques enables analytical laboratories to ensure high accuracy while supporting more sustainable, green chemistry validation practices.

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